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CORK FORMATION IN THE ROOTS OF MEDICAGO AND MELILOTUS IN RELATION TO DISEASE DEVELOPMENT

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University of Alberta
April, 1931

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CORK FORMATION IN THE ROOTS OF MEDICAGO AND MELILOTUS IN RELATION TO DISEASE DEVELOPMENT.

Melville Wallace Cormack
Department of Field Crops

A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements for
the degree of

MASTER OF SCIENCE

Edmonton, Alberta
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TABLE OF CONTENTS.

Page

Introduction.................................... 1

Literature review................................ 3

Studies on other plants......................... 3

Factors influencing cork formation.............. 7

Cork formation in the roots of Medicago......... 11

General experimental procedure.................. 13

Material.. 13

Treatment....................................... 16

Histological technique.......................... 17

Experimental results............................ 19

Studies on young, growing plants................ 19

Controlled experiments:- The influence of soil temperature and moisture on wound cork formation.......................... 20

Field experiments: The influence of soil temperature on wound cork formation in relation to disease development............ 25

Studies on overwintered plants.................. 33

Influence of temperature on cork formation and disease development in overwintered plants.......................... 34

Influence of soil temperature and moisture on cork formation and disease development in artificially frozen plants............... 40

Page

Cork formation and disease development in plants of the second year's growth.......... 44

General histological studies........................ 48

Histological features of normal alfalfa and sweet clover roots at different stages of growth................................... 48

Manner of wound cork formation in the different root tissues...................... 50

Pathogene penetration and development in the root tissues.......................... 53

General Discussion and Conclusions.................. 61

Summary.. 69

Acknowledgment................................... 71

References.. 72

CORK FORMATION IN THE ROOTS OF *MEDICAGO* AND *MELILOTUS* IN RELATION TO DISEASE DEVELOPMENT.

Melville Wallace Cormack.

INTRODUCTION

Cork formation plays an important rôle in disease resistance in many plants. Its possible important relation to disease development in the roots of alfalfa and sweet clover was suggested during studies on the root-rot problem of these crops. This appeared to offer a promising field of study since preliminary observations indicated that cork was formed quite readily in these roots. The following investigation was accordingly undertaken with the primary object of determining the relation, if any, of cork formation to disease development in the roots of alfalfa and sweet clover.

The influence of certain factors on cork formation was also studied. These factors have been shown to exert a marked influence in several other plants. The temperature relation was very important since alfalfa and sweet clover roots are most severely damaged when the temperature is relatively low, following the winter dormancy period. A possible retardation of cork formation at that time was suggested. Unfavorable soil moisture

relations might also retard growth processes in the roots following the dormancy period. Another factor considered was the age and condition of the plants at the time when they were attacked by the root-rotting organisms. These investigations were carried out under more or less controlled conditions in the greenhouse and cold rooms and also under natural growing conditions in the field.

The importance of root-rotting diseases and winter killing of alfalfa and sweet clover is emphasized by the rapidly increasing cultivation of these crops in western Canada. It was formerly thought that the partial or complete destruction of the plants, following the winter dormancy period, was entirely due to frost injury or lack of winter hardiness. The frequent association of certain pathogenic root rotting organisms with winter killed plants has led to the present belief that they are at least partially responsible for winter killing.

In the preliminary studies of Newton and Brown (18) in 1924, root lesioning and rotting, apparently caused by some species of _Sclerotinia_, was found on the majority of winter killed plants. No winter injury was observed on plants growing in boxes of sterilized soil. In 1929, Sanford (22) reported the isolation of a definite soil organism which caused serious damage to alfalfa, sweet clover and related crops in the spring, following over-wintering. This organism was named _Plenodomus meliloti_ Dearness and Sanford. It has a very low temperature relation as indicated by its growth at 0° C., and pathogenicity

tests show that only plants which have passed through the winter dormancy period are attacked. The damage caused by Sclerotinia sp. is more severe during warmer weather but may occur at any time during the growing period. Both organisms undoubtedly cause serious losses by attacking the roots of alfalfa and sweet clover.

Preliminary observations and tests have indicated differences in varietal susceptibility to the attack of these organisms. Sanford (22) found that Grimm alfalfa and Arctic sweet clover were apparently more resistant than other varieties to the attack of Plenodomus meliloti under field conditions. Kirk (15) also reported that Arctic sweet clover was more winter hardy and showed resistance to root-rotting organisms. It was hoped that this investigation would either prove or disprove the hypothesis that cork formation was concerned in the prevention of disease development in the roots of alfalfa and sweet clover.

LITERATURE REVIEW

Studies on Other Plants.

Cork is extremely resistant to the action of micro-organisms and has proved to be the cause of disease resistance in many plants. The rapidity and extent of cork formation may signify plant varietal resistance to pathogene invasion. In other plants it may be only a contributing factor, or of no significance in

disease resistance. The tissues of some plants are unable to form cork and it is of no significance in a few plants which are potentially capable of cork formation. It is of relatively little importance in monocotyledonous plants (19) and has never been reported in the family Gramineae, which includes many important crop plants.

A search of the literature has revealed very little previous work on cork formation in Medicago, Melilotus or related plants. Accordingly a general review of studies in other plants will be given, laying special stress on cork formation in roots and subterranean plant parts.

The common potato has been most extensively studied. Appel (1) found that the potato was capable of closing a wound within a short time by cork formation. This newly formed cork layer was usually able to wall out the bacterium causing potato black leg, but was sometimes penetrated by fungi. Leach (16) concluded that the invasion of the black leg organism would have been entirely checked if it had not been able to invade the vascular bundles, which were unable to form cork. An effective cork wall was, however, laid down in the tissues surrounding the bundle, thus preventing escape of the bacteria for some time. After removal of the starch by the sprout, the seed piece was no longer able to form cork and the bacteria escaped and proceeded to spread through the tissues and into the stem.

Fellows (10) found that potato scab infection took place through stomata or young uncorked lenticels at a very early

stage of tuber growth. The typical corky scab lesions were formed only when actively dividing cells or those which could be readily incited to division were present. Lutman (17) believed that the thickness of the normal cork layer determined the resistance of tubers to the potato scab organism. He found that true russet types of tubers with thick corky skin layers were more resistant than thin skinned varieties.

The importance of cork formation in the healing of cut potato seed pieces was emphasized by Priestley and Woffenden (20). Shapovalov and Edson (23) found that decay spread more rapidly in the central portion of the seed piece, where the initiation of cork formation was delayed. Weiss et. al. (30) in studies on Fusarium rot in stored potatoes, concluded that the most important factors in preventing infection were those influencing cork formation.

Weimer and Harter (29) studied wound cork formation in the sweet potato. A poorly developed cork layer was produced beneath wounds under conditions existing in the storage house. A hard surface covering on the wound, however, appeared to be a fairly efficient barrier against the invasion of micro-organisms.

Conant (7) studied resistance to the root-rot organism, Thielavia basicola, in tobacco. Cork formation was very rapid and pronounced in the roots of resistant varieties and effectively walled out the pathogene under favorable conditions. In susceptible varieties, both normal and wound cork were much slower in forming and offered little resistance to invasion

of the pathogene into the tissues. The cork layers of resistant varieties were very resistant to invasion, being penetrated only by mass action of the fungal hyphae. This was generally followed by the formation of a new, deeper cork layer.

Tisdale (25) reported varietal resistance in flax to Fusarium lini; the organism causing flax wilt. In resistant flax, a cork layer was formed in the roots between the infected outer portion and the vascular tissues, which prevented further spread of the organism. Cork formation was not initiated in susceptible plants. This author, however, suggested that substances were present in resistant roots which retarded or weakened the fungus, thus aiding the effectiveness of the cork.

Smith and Walker (24) studied resistance to yellows in cabbage roots. They concluded that the resistance observed in certain varieties and strains was not due to morphological causes, since wound cork formation or other cell changes were not observed.

The studies of Wardlaw (26) on banana wilt, caused by Fusarium cubense, showed that a cork layer was formed in all parts of the root cortex in response to fungous invasion. This cork prevented deeper penetration of the fungus into the cortex and stele except in cases where diseased rootlets passed through the cortex. Older roots were more resistant.

The significance of cork formation in the aerial parts of plants has also been studied. Trees with a corky bark covering are commonly observed to be more resistant to disease

(11). Wiltshire (31) showed that the fungus causing apple canker was excluded from the interior of the host by cork formation, unless it had already reached the wood cells. Coutant (8) reported an unusual type of wound cork formation in certain cacti; new vascular bundles being eventually formed.

Cork formation often aids in the healing of wounds in leaves. Wylie (32) found that cork formation around the border of a leaf wound was very effective in preventing infection. Butler (6) reported that the lesion caused by the citrus scab organism is often walled off by cork. Samuel (21) and Blackmann and Matthaei (4) found cork formed around lesions in leaves of various plants, which generally resulted in the "shot hole" effect.

Bartholomew (3), in studying *Alternaria* rot of lemons, found that layers of cork were formed under the button of the fruit in advance of the invading pathogene. As many as three successive layers might be formed, which were usually effective in excluding the pathogene. Valleau (27) reported varietal resistance to brown rot in plums. In most cases this was directly correlated with the efficiency of the cork protection in the stomata and lenticels.

Factors Influencing Cork Formation.

Several investigators have noted the marked influence of certain factors in determining the initiation, rapidity and extent of wound cork formation in plants. The more important factors

are temperature, humidity, and the age and variety of the plant. Cork formation may be stimulated, retarded or completely inhibited under certain conditions. These factors may, therefore, have a great influence in determining the significance of cork in relation to disease development.

The early initiation and the rapidity of cork formation after wounding are of importance in excluding pathogenic organisms. Appel (1), and Artschwager (2) concluded that the rapidity of wound cork formation was more important than the thickness of the cork layer. Other studies (5,7) indicated that this is often a determining factor in disease resistance. Resistant varieties are usually capable of laying down cork walls more rapidly, but this ability is often profoundly influenced by a number of other factors.

Temperature is known to exert a marked influence on cork formation. It is generally retarded by low temperatures. In the common potato, Priestly and Woffenden (20) found that the rate of cork formation in cut tubers was doubled when the temperature rose from 15° C. to 25° C. Artschwager (2) reported that the delayed suberization of the cut tuber surface at low temperatures resulted in delayed cork formation in the deeper tissues. Cork cells were first formed in tubers of the Irish Cobbler variety at 7° C., after the ninth day, but none were formed at lower temperatures. They appeared after the sixth day at 10° C. and after the fourth day at 15° C. Several layers of wound cork cells were formed after the third or fourth day

at this temperature. Other varieties formed cork more slowly at the lower temperatures, but all were equal at 15° C. Weiss et al (30) concluded that the effect of temperature on cork formation and other wound healing processes in stored potatoes overshadowed the direct effects of the environmental factors, temperature, humidity and aeration, on the progress of storage rot organisms. At 10° C. none of the varieties studied were able to form a protective cork layer beneath wounds in less than 10 days. This would, however, exclude the more virulent storage rot organisms. Wound cork formation was so greatly delayed at 6.7° C. that there was no obstacle to infection.

Weimer and Harter (29) found that temperature had an important influence on wound cork formation in the sweet potato. A protective cork layer was formed quickly in wounds at temperatures between 26° C. and 33° C., while the process was greatly retarded below 20° C. A well developed cork layer was not produced under conditions existing in the storage house.

In studies on root-rot resistance in tobacco, Conant (7) found that the lower the temperature at which a tobacco root could initiate normal, premature or wound cork formation, the greater was its resistance to the causal organism. At 20° C. and over, cork was consistently formed in response to fungous invasion in the resistant variety, Xanthia, and was stimulated in all varieties at 25° C. At 28° C., the initiation of phellogen activity occurred almost as early in susceptible varieties as in resistant varieties at 20° C.

Brooks (5) reviewed investigations on larch and apple canker. The causal fungi progressed rapidly in the bark during the winter, but were temporarily checked in the spring by the formation of a cork barrier, indicating that temperature might have an important influence.

Moisture has a pronounced effect on cork formation in some plants. Studies on this relation have been practically confined to atmospheric humidity and no reference as to the effect of soil moisture has been found in the literature.

Artschwager (2) found that cork formation in cut potato tubers was favored by high atmospheric humidity. At 12° C. cork was formed only in tubers held at 94 per cent relative humidity. Even at 6.5° C. a well developed cork layer was formed in 53 days under these conditions. Unexplainable irregularities in cork formation were obtained. Priestley and Woffenden (20) showed that a continuous cork layer was formed when the cut surface of a potato tuber was exposed in a moist atmosphere, but the layer was often discontinuous in a dry atmosphere. They recommended that cut potato sets should be kept under cool, moist conditions and not exposed to strong sunlight before planting. Weiss et al (30) found that wounds healed more rapidly in relatively moist tubers, preventing the growth of storage rot organisms.

In sweet potatoes, Weimer and Harter (29) showed that a relative air humidity of 95 to 100 per cent was most favorable for cork formation. The absence of cork in tuber wounds, under

storage conditions, was attributed largely to the low humidity.

A different moisture relationship has been demonstrated in leaves. Samuel (21) studied fungous invasion in almond leaves and found that wound cork was formed under dry air conditions and checked the progress of the fungus. Abscission occurred under relatively moist conditions. Blackmann and Matthaeii (4) also noted that relative dryness of the surrounding air appeared to be the essential cause of cork formation, rather than abscission, in the wounded leaves of a number of trees.

The age and condition of plants or plant parts may influence cork formation. Fellows (10) concluded that potato scab infection took place through uncorked lenticels or stomata at a very early stage of tuber growth. No infection occurred when the tuber ceased growth and was completely protected. Wardlaw (26) reported that young banana roots showed a rapid dying back on inoculation, but that older roots resisted penetration by suberization, cork formation and toxic secretions.

Conant (7) found that tobacco plants showed a gradient of host reaction, as expressed by cork formation, which agreed closely with the general vigor of the plant. Brooks (5) states that the fungus causing larch canker is successfully walled out by cork barriers, especially in vigorously growing larch trees.

Cork Formation in the Roots of Medicago.

No reported investigations on cork formation in sweet clover

have been found in the literature. It has been studied in alfalfa roots in connection with investigations on winter killing and disease invasion. Jones (12) studied frost injury in alfalfa roots in relation to the histology of the cells. Growth response to injury was found to be conditioned by the character of the adjoining cell tissue. Decay did not proceed far beyond the cells originally killed, if these were capable of prompt meristematic activity. All parenchymatous tissue appeared to be capable in varying degree of laying down cork cells, except certain wood ray cells which had become considerably elongated axially.

Injury in the phloem and in the central pith-like parenchyma met with the quickest response in surrounding cells. A new cork layer similar to the outer covering was produced by the actively dividing phellogen. This layer was usually broken and ineffectual when it arose in the immature cells near the cambium. In the mature phloem it was often interrupted by groups of fibres. These fibres pursue a sinuous course through the tissues and allowed decay to traverse the cork layer into the underlying cells. These cells usually divided rapidly enough to wall out the decay, but in so doing often ruptured the outer covering of the root.

In later investigations Jones (13) studied the development of wilt injury in alfalfa roots caused by the bacterium, Aplanobacter insidiosum L. Mc. Spring infection was most common and this was found to be due to the entrance of the bacteria through

wounds caused by winter injury. They usually advanced unhindered throughout the tissues. No mention was made of cork formation in relation to invasion and the author's illustrations do not give any evidence of its formation.

Weimer (28) also studied alfalfa root injuries resulting from freezing. The ability of alfalfa roots to heal their injuries was greater in the first year of growth. Almost all tissues of the root were shown to be potentially meristematic in nature. Large dead areas in either the phloem or the centre of the root were cut off by meristematic activity from the living tissues beneath. The greatest injury was found in the phloem. A layer of cork was formed separating the injured from the uninjured portion but this layer was irregular and unequal in thickness. The author did not consider this cork layer as being a very effective barrier against the invasion of pathogenic organisms, at least not equal to the normal cork layer at the outside of the root. These histological studies did not disclose any criterion for distinguishing between hardy and non-hardy varieties of alfalfa.

GENERAL EXPERIMENTAL PROCEDURE.

Material.

The varieties of alfalfa and sweet clover used in this investigation were selected on the basis of contrasting hardiness.

Relatively hardy and non-hardy varieties were studied in order to determine any possible differences in disease resistance and cork formation. Among the following varieties utilized in the different experiments; Grimm alfalfa and Arctic sweet clover are known to be distinctly hardier than the others:-

Alfalfa:- Medicago media: Grimm, Cossack, Liscombe and Baltic.
Sweet Clover:- Melilotus alba: White Blossom, Arctic and Maccor.
Melilotus officinalis: Yellow Blossom.

During the course of this investigation, plants in different stages of growth were used, in order to determine whether the age and condition of the plant had any bearing on disease development and cork formation. The following four main types of plants were studied:-

1. Young plants in the summer and fall of the first year's growth.
2. Overwintered plants transplanted from the field before thawing in the spring.
3. Mature plants of the second year's growth.
4. Plants grown in the greenhouse under relatively unfavorable growth conditions.

In the studies on disease development, two different root-rotting fungi were employed for artificial inoculation on the roots of the different varieties. These organisms were of proved pathogenicity on alfalfa and sweet clover and possessed contrasting types of growth and temperature relations. The following general disease symptoms are produced by each organism;

Plenodomus meliloti Dearness and Sanford . This recently

identified soil inhabiting organism causes the brown root-rot disease of alfalfa and clovers. It has proved to be highly pathogenic to both alfalfa and sweet clover, but artificial field infection has been produced only in the spring following the winter dormancy period. It thrives at very low temperatures, growing at 0° C. in artificial culture, with an optimum of 15° C. It is believed to progress in the root tissues and cause rotting from late winter until June, at which time the lesions may heal, if the plant has not been killed. Brown rotted areas occur on the tap and lateral roots and may involve the crown. Characteristic small black fruiting bodies or pycnidia are found upon and within the rotted tissue. The culture used in this study was isolated from a diseased sweet clover root.

Sclerotinia trifoliorum Erikks. causes the disease known as Sclerotinia root-rot and is highly pathogenic on alfalfa and sweet clover. Large or small lesions appear on the tap root and laterals, or the root may be completely rotted and the plant killed. This may occur at any time during the growth of the plant, but is usually more pronounced following the winter dormancy period. Hard, black sclerotial masses are often formed in the diseased tissues. The culture used in this study was isolated from a diseased sweet clover root. Positive species identification has not been made, but it resembles *Sclerotinia trifoliorum* in general characteristics. This strain proved to be much more pathogenic on sweet clover than on alfalfa.

Treatment.

The plants used in the controlled experiments performed in the greenhouse and cold rooms were usually grown in the field and transplanted into boxes or crocks. In field studies the treated plants were kept as closely as possible under normal growing conditions. The soil temperature was recorded in all experiments by means of soil thermographs.

A general method for inoculation and treatment of the roots was developed and used in most of the experiments. Each tap root was first bared of earth on one side to a depth of 3 - 4 inches and brushed reasonably clean. The main branch roots were injured as little as possible. By means of a sharp scalpel, two cuts were made on the root at a depth of about 2 inches from the crown. A surface cut, which removed a thin slice of the outer portion of the root was made in one place, and a little to one side of this a deep slit was made, which extended from the outside to the centre of the root.

Each root was then inoculated with one of the organisms described, at a depth of between one and two inches below the cuts. A small portion of oat hull inoculum of the organism was placed on a thin piece of cotton batting and packed firmly against the root at this point. In some cases the root was cut at the point of inoculation and in others left uninjured. After inoculation the earth was replaced and firmed down.

The roots were removed from the soil at varying times

after treatment for examination. Notes were taken on the diseased portion at that time. An arbitrary numerical infection rating was placed on each root. This varied from 0 - 10; in which 0 indicated freedom from infection and 10; very severe infection, which usually resulted in death of the plant. The inoculated and non-inoculated portions of the roots were then either sectioned immediately or preserved for future histological examination.

Histological Technique.

All material was preserved and fixed in form-acetic fixing solution. This consisted of formaldehyde - 10 parts, acetic acid - 5 parts and water - 85 parts. Whenever possible, histological examination was first made while the roots were in the fresh condition. When this was inconvenient, they were fixed, and sectioned at a later date, after washing in running water for 12 hours.

A rough sectioning method was used for the first examination of all material. A small hand microtome was used for holding the roots and sections were cut with a sharp sectioning razor. These sections varied from 25 μ to 75 μ in thickness, depending on the condition of the material. They were floated in water until stained.

A staining technique was finally worked out which gave very good results. The rough sections were first boiled in Sudan III (95% alcoholic solution) on a glass slide over a bunsen burner for 1 - 2 minutes. Immersion in the stain for 12 hours

gave equally good results. They were then floated in distilled water for 5 minutes, followed by immersion in aqueous Acid Fuchsin for 5 minutes and in an aqueous solution consisting of Light Green-9 parts; Iodine Green-1 part for 10 minutes. The sections were washed between stains and destained at the end in distilled water. They were permanently mounted, when desired, in gum arabic - sugar mounting medium. This method gave a very good differentiation, staining cork cells - orange; diseased tissue and fungous mycelium - pink to red, and the normal tissue - blue.

Notes were taken on cork formation and disease development in these sections, as revealed by microscopic examination. An arbitrary numerical cork rating was used in estimating cork formation. The numbers 0 - 10 were used to represent varying degrees of cork formation on the following basis:

0-None.	No trace of phellogen formation.					
1-Trace.	Scattered cork cells.				Weakly	suberized.
2-Light-	Fairly continuous layer,	1	cell wide	(5µ-10µ)	"	"
3-Light	" " "	1	" "	(10µ-15µ)	"	"
4-Light+	" " "	1	" "	(15µ-20µ)	Fully	suberized.
5-Medium-	Continuous layer,	2	cells wide	(20µ-25µ)	"	"
6-Medium	" "	2	" "	(25µ-30µ)	"	"
7-Medium+	" "	2-3	" "	(30µ-35µ)	"	"
8-Heavy-	" "	3	" "	(35µ-45µ)	"	"
9-Heavy	" "	4	" "	(45µ-50µ)	"	"
10-Heavy+	" "	4+	" "	(50µ+)	"	"

This method showed some of the material to be of particular

value for more careful examination and photographing. This was sectioned after infiltration with paraffin. A butyl alcohol series through which the material could be run very quickly was used for dehydration. The sections were cut 10 - 12µ thick on a sliding microtome. The same staining technique was used as with the rough sections with the exception that the slides were placed in Sudan III for 12 hours or more prior to using the other stains. The sections were permanently mounted and labelled.

EXPERIMENTAL RESULTS.

Studies on Young, Growing Plants.

It was thought advisable to study cork formation and the various factors which influence it in young growing plants, before attempting to interpret its significance in overwintered plants. There was a possibility that retarded cork formation in the roots following exposure to low temperatures might be responsible for the increased susceptibility of the plants to root-rotting organisms at that time. It was thought that moisture as well as temperature might be important, as both of these factors have been shown to have a marked influence on cork formation and disease development in many other plants. These factors were studied, using young alfalfa and sweet clover plants kept in the greenhouse and cold-rooms under

artificially controlled conditions or grown in the field under natural conditions.

Controlled experiments:- The influence of soil temperature and moisture on wound cork formation. The object of these experiments was to study the influence of varying soil temperatures and moistures on cork formation and its relation to disease development in the roots of alfalfa and sweet clover. The effect of time, variety of the host and nature of the pathogene on cork formation were also considered. The experiments were carried out under carefully controlled artificial conditions, in the greenhouse and cold rooms.

The following varieties of alfalfa and sweet clover from the 1930 field planting were used:

Alfalfa - Grimm and Cossack.
Sweet clover - White Blossom and Arctic.

The plants were grown from seed planted on June 27th. They made a very vigorous growth during the summer. When transplanted on August 30th, the roots averaged about one-half inch in diameter at the crown.

These plants were transplanted directly from the field into wooden boxes. Twenty four boxes, 2 1/2 feet long, 1 foot wide and 1 foot deep were constructed for the purpose. The top board on each box was made easily removable, to allow convenient treatment of the roots at a later date. After filling with field soil, 20 plants were transplanted into each box. A row of 10 plants of a single variety was planted on one side of a

box with a similar row of another variety on the other side. In this way 12 boxes each of alfalfa and sweet clover were prepared. Care was taken in transplanting to avoid unnecessary injury of the roots. The boxes were left in the field for two weeks to allow recovery from the effects of transplanting. They were then taken inside and divided up for placing under controlled conditions. An equal number of boxes was kept at each of three temperatures; 1° C., 9° C. and 18° C. Two boxes each of alfalfa and sweet clover, kept dry, and a similar number of each, kept moist, were exposed to each temperature.

A soil temperature of approximately 18° C. was maintained in the greenhouse by controlling the air temperature. An average soil temperature of 18.3° C. was obtained for the whole period with a maximum variation of 4° C. on either side. The two lower temperatures were secured in insulated cold rooms refrigerated by an ammonia plant. The boxes of plants in the cold rooms were first hardened off by gradually lowering the temperature. After about a week, the soil temperatures were finally adjusted to approximately 1° C. in one room and 9° C. in the other. The air temperature, automatically controlled by a thermo-regulator, showed a maximum variation of 2° C. on either side. This kept the soil temperature quite constant. Soil thermographs were used to record all soil temperatures.

The soil moisture was roughly regulated by keeping the dry boxes at a minimum soil moisture content for normal growth and the other boxes relatively wet. The moisture content of the soil in all boxes was determined at regular intervals and

kept reasonably constant. The average moisture content of the dry soil was 21.5 per cent of its dry weight and of the wet soil, 30.2 per cent of its dry weight.

All roots were both inoculated and cut, using the general methods described. The plants in the cold rooms were inoculated with Plenodomus meliloti and those in the greenhouse with Sclerotinia trifoliorum. The plants were all in excellent condition at the time of treatment, those in the cold rooms being supplied with light from 200 watt bulbs burning continually.

Four replicate plants were taken up from all varieties and treatments, at intervals of 8, 13, 18, 23 and 28 days after treatment. After taking notes on the condition of the roots, they were preserved for histological examination. These roots were later cut and stained as previously described.

The results obtained in this experiment are shown in Table I. The figures representing cork formation were based on the thickness, regularity and degree of suberization of the wound cork layer in non-inoculated cuts, at the time of removal of the roots from the soil. They represent the average rating of four plants and are purely relative. Since very little infection was obtained with either organism, no conclusions could be drawn on the relation of cork formation to disease development.

At 1° C. cork formation was not initiated at any time during the course of the experiment. Even after 28 days there

Table I. Effect of soil temperature[a] and moisture on wound cork formation in different varieties of alfalfa and sweet clover.

Variety	Soil Moisture	Cork Rating[b] at Different Periods after Treatment														
		8 days			13 days			18 days			23 days			28 days		
		1°	9°	18°	1°	9°	18°	1°	9°	18°	1°	9°	18°	1°	9°	18°
Grimm Alfalfa	Dry	0	0	2.2	0	0	5.9	0	0.9	6.6	0	2.4	7.7	0	3.4	8.0
	Moist	0	0	0.6	0	0	4.2	0	0	6.6	0	0.9	6.8	0	5.0	7.2
Cossack Alfalfa	Dry	0	0	2.4	0	0	5.2	0	0.9	6.2	0	2.0	8.0	0	2.4	8.1
	Moist	0	0	1.0	0	0	4.7	0	0.1	7.0	0	0.9	7.4	0	2.5	7.8
White Blossom Sweet Clover	Dry	0	0	2.9	0	0	4.5	0	0.8	5.9	0	2.7	7.5	0	2.9	8.3
	Moist	0	0	1.8	0	0	5.1	0	0.3	6.2	0	1.7	7.4	0	3.5	7.6
Arctic Sweet Clover	Dry	0	0	4.8	0	0	5.8	0	1.2	7.9	0	1.9	8.1	0	3.1	8.5
	Moist	0	0	3.5	0	0	4.5	0	0.4	8.0	0	0.6	8.3	0	1.7	8.6

a. Soil temperatures in degrees Centigrade.

b. Average numerical rating of four replicates.

was no trace of a phellogen layer in the tissues and the cells at the cut surfaces were still ruptured and loose. There is of course a possibility that cork formation might still occur at a later time, but the unchanged character of the tissues indicates complete inhibition. At 9° C. the first signs of cork formation were seen in 18 days after which it progressed slowly. In 28 days, a fairly continuous layer of cork cells occurred beneath most cuts but this layer was never more than one cell thick and often poorly suberized. At 18° C. cork formation was initiated prior to 8 days and fairly continuous layers were formed at this time. In 28 days, a very regular, well suberized cork layer, 2 - 3 cells wide was formed beneath most cuts. These results indicate that temperature is the predominating factor controlling cork formation in these plants. Cork formation was distinctly inhibited or retarded at low temperatures.

Soil moisture had no striking effect on cork formation under the conditions of this experiment. The results show that cork formation was initiated slightly earlier under moist soil conditions, but they are by no means conclusive. The slight differences shown in the early stages of cork formation apparently disappear in time. Unexplainable variations within replicates were often obtained.

No regular or significant varietal differences in cork formation were observed. Arctic sweet clover roots, however, generally had a more regular and sometimes thicker cork layer

beneath the wounds.

The almost complete failure of infection in inoculated roots was the most disappointing feature of this experiment. The failure of *Plenodomus meliloti* to attack the roots was not surprising since this organism has not previously been reported as attacking roots which have not overwintered. As far as is known conditions were favorable for the development of *Sclerotinia trifoliorum* and no explanation can be offered for the failure of infection in this case. Only occasional cases of root-rotting occurred, which were of no significance.

Field experiments: *The influence of temperature on wound cork formation in relation to disease development.* The object of this experiment was to study cork formation in relation to disease development, under normal growing conditions in the field. It was hoped that infection of young, growing alfalfa and sweet clover roots could be obtained under these conditions, so that its relation to cork formation could be determined. A number of different varieties were used in order to determine any possible differences in cork formation or disease reaction. The influence of soil temperature was studied by conducting the experiment twice in two different months of contrasting temperature. The first experiment was carried out during September when the soil temperature was relatively high. The weather was quite warm until towards the end of the month. A similar experiment was conducted during October when the soil temperature was relatively low. A

snowstorm and very cold, wintry weather occurred during this month. It was impossible to study the influence of soil moisture satisfactorily under field conditions.

The following varieties of alfalfa and sweet clover were used in both experiments:

Alfalfa: Grimm, Cossack, Baltic and Liscombe,

Sweet Clover: White Blossom, Arctic, Maccor and Yellow Blossom.

The plants were grown from seed planted on June 27th in the 1930 field planting. An excellent stand, with good sized roots, was secured by the end of August when the first inoculations were made.

The two root-rotting organisms: Plenodomus meliloti and Sclerotinia trifoliorum were used for inoculation. The inoculum was applied to each root on a thin piece of cotton batting as described in the general methods. About half the roots were cut at the point of inoculation and the remainder left uninjured, in order to determine the mode of fungal penetration. Twenty four plants of each variety were inoculated with each organism and the same number cut for non-inoculated checks. All plants were disturbed or injured as little as possible when making treatments.

Four plants from each variety and treatment were removed for examination at the end of periods 5, 10, 15, 20, 25 and 30 days after treatment. Notes were taken on disease development and a histological examination made of the fresh roots when possible. They were cut and stained by the general technique

previously described. All roots were preserved in form-acetic fixing solution.

The soil temperature for both periods was recorded by means of a soil thermograph. Treatments for the first experiment were made on August 26th and the last plants taken up on September 24th. The mean soil temperature for this entire period, based on daily averages, was 11° C. The maximum temperature recorded was 18° C. and the minimum 5° C. which occurred toward the end of the experiment. The second experiment extended from October 7th to November 5th. The mean soil temperature for this period was 1.1° C. The maximum temperature recorded was 6.6° C. and the minimum 0° C., but the plants were never frozen. Very little variation occurred in soil temperature over most of the period.

The influence of soil temperature on cork formation in these two periods is shown in Table II. No cork formation was observed in any of the wounded roots under the low soil temperature conditions prevailing in the October period. Even in 30 days, there was no indication of possible subsequent phellogen development. These results confirm the observations made in the controlled experiment, on the inhibition of cork formation at low soil temperatures.

Cork formation was first observed in 10 days following treatment, under the fairly high soil temperature conditions of the September period. In non-inoculated roots a fairly continuous cork layer, 1 cell thick, was formed under most cuts at that time. Cork formation progressed very rapidly until in 20 days, a very regular, unbroken cork layer, 2 — 3 cells

Table II. Effect of field soil temperature on wound cork formation in the roots of alfalfa and sweet clover inoculated and non-inoculated with Sclerotinia trifoliorum.

Variety	Treatment	Cork Rating[a] at Different Periods after Treatment											
		5 days		10 days		15 days		20 days		25 days		30 days	
		1°	$11^{\circ b}$	1°	11°	1°	11°	1°	11°	1°	11°	1°	11°
Grimm Alfalfa	Inoculated	0	0	0	0.3	0	3.3	0	7.2	0	4.7	0	8.3
	Non-inoculated	0	0	0	3.1	0	3.3	0	8.8	0	7.3	0	7.8
Cossack Alfalfa	Inoculated	0	0	0	0.2	0	1.6	0	6.0	0	4.7	0	4.8
	Non-inoculated	0	0	0	2.8	0	6.6	0	7.5	0	6.2	0	8.9
Baltic Alfalfa	Inoculated	0	0	0	0	0	1.3	0	3.3	0	3.0	0	5.7
	Non-inoculated	0	0	0	2.2	0	5.3	0	7.5	0	7.2	0	7.3
Liscombe Alfalfa	Inoculated	0	0	0	0	0	0	0	3.0	0	3.0	0	7.0
	Non-inoculated	0	0	0	1.5	0	3.3	0	7.7	0	7.0	0	9.0
White Blossom Sweet Clover	Inoculated	0	0	0	0	0	0	0	0	0	2.3	0	1.5
	Non-inoculated	0	0	0	3.8	0	7.0	0	7.2	0	7.5	0	7.5
Arctic Sweet Clover	Inoculated	0	0	0	0	0	0	0	0	0	0	0	0
	Non-inoculated	0	0	0	4.1	0	6.6	0	6.6	0	6.8	0	6.0
Maccor Sweet Clover	Inoculated	0	0	0	0	0	0	0	0	0	0	0	0
	Non-inoculated	0	0	0	2.3	0	7.2	0	7.0	0	8.0	0	5.3
Yellow Blossom Sweet Clover	Inoculated	0	0	0	0	0	0	0	0	0	0	0	0.8
	Non-inoculated	0	0	0	4.6	0	6.0	0	8.8	0	7.2	0	6.8

a. Average numerical rating of four replicates.

b. Mean soil temperatures in degrees Centigrade for October and September, respectively.

Table III. Development of *Sclerotinia trifoliorum* in alfalfa and sweet clover roots at varying soil temperatures in the field.

Variety	Infection Rating[a] at Different Periods after Inoculation											
	5 days		10 days		15 days		20 days		25 days		30 days	
	1°	11°[b]	1°	11°	1°	11°	1°	11°	1°	11°	1°	11°
Grimm Alfalfa	0	1.1	0.1	1.3	0.6	0.7	0.5	1.8	2.3	0.7	1.8	2.8
Cossack Alfalfa	0	0.3	0.1	1.2	0.3	1.5	0.7	1.4	0.6	2.0	2.3	2.2
Baltic Alfalfa	0	1.0	0.3	1.6	0.4	1.3	0.4	0.7	1.6	1.3	1.7	0.8
Liscombe Alfalfa	0	1.3	0.4	1.5	1.0	0.3	1.5	0.2	4.0	2.0	3.5	0.2
White Blossom Sweet Clover	0.1	2.0	1.5	8.8	5.1	9.0	4.9	8.3	5.8	9.0	7.8	9.5
Arctic Sweet Clover	0	1.7	1.1	7.3	3.7	3.0	2.5	6.3	2.6	7.0	5.2	8.3
Maccor Sweet Clover	0	1.4	1.5	6.7	4.0	9.0	6.0	7.5	3.5	6.5	8.5	7.5
Yellow Blossom Sweet Clover	0.1	2.3	2.7	6.0	4.0	6.6	4.2	6.5	4.4	7.1	6.7	7.8

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[a] Average numerical rating of four replicates.

[b] Mean soil temperature in degrees Centigrade for October and September, respectively.

wide, had been formed beneath most cuts. The field soil temperatures, even in the first 10 days, were decidedly lower than the greenhouse soil temperature of 18° C. used in the controlled experiment. This indicates that cork is formed more readily at lower temperatures, under natural growing conditions in the field, since the cork layer was equally well developed in the same time, under both field and greenhouse conditions. There was consistently less cork formed in inoculated than in non-inoculated roots, which will be discussed later in connection with disease development.

Inoculation with *Plenodomus meliloti* again resulted in an almost complete absence of infection of the roots. A few slightly lesioned roots, however, were obtained in both periods and the fungus was reisolated from a lesion in one case. At the higher temperature, root lesioning apparently caused by drought, often occurred beneath the cotton batting on which the inoculum was placed. This made it difficult to distinguish true fungous lesions. There was some indication, however, that this organism might cause infection in young growing roots, under suitable conditions.

The development of *Sclerotinia trifoliorum* in the inoculated roots is shown in Table III. The most interesting feature of these results was the marked resistance of all varieties of alfalfa to the attack of this organism. This resistance was quite constant with the strain of *Sclerotinia* used in the experiment. In 5 days at the higher temperature, most alfalfa

roots had been slightly attacked by the fungus. The small surface lesions formed, however, never increased in size throughout the course of the experiment. The organism was apparently checked in some manner, shortly after invading the tissues. Cork formation could not have checked the invasion of the pathogene, since no cork was formed in these roots in 5 days and the cork layer was very broken in 10 days, even in non-inoculated roots. It was 20 days after inoculation before a cork layer of any depth was laid down beneath the small lesions and this was often broken and apparently ineffectual in preventing further invasion of the fungus.

Similar results were obtained in the October experiment at a lower soil temperature. In this case the small lesions were not very noticeable until 15 days and made no progress after that time. No cork was formed at any time during the experiment, which further substantiated the fact that cork formation played no part in checking the fungous invasion.

The inoculation of sweet clover roots with Sclerotinia gave very different results, which were most marked in the September experiment. The fungus caused distinct rotting in 5 days and progressed at an amazing rate in the tissues in the period between 5 and 10 days after inoculation. In 10 days, the tops of a number of plants were dead and the roots completely rotted. White Blossom sweet clover was most severely attacked, the majority of the roots being rotted

through after 10 days, as shown by its high infection rating in Table III. The plants of other varieties were seldom killed but severe root-rotting occurred in all cases.

In the October experiment, fungous invasion of the sweet clover roots was noticeably retarded by the lower soil temperatures. Small lesions did not appear until 10 days after inoculation in most cases. The fungus progressed fairly rapidly in the tissues, but even in 30 days, the roots were not as severely rotted as in 10 days at the higher soil temperature. The relatively low infection in the roots of Arctic sweet clover was the only constant difference in varietal reaction observed in this experiment.

Cork formation was apparently not responsible for these varietal differences of sweet clover in disease reaction. As shown in Table II, no constant differences were observed in cork formation in either inoculated or non-inoculated roots of the different varieties. In cases where roots were badly rotted, they were examined for cork formation at points where there was little rotting. No cork was formed at the low soil temperatures of the October experiment. In the September experiment, a marked and consistent difference occurred in cork formation in inoculated and non-inoculated roots. In Arctic and Maccor sweet clover no cork was observed at any time beneath the lesions and only an occasional broken layer occurred in the other varieties. This was undoubtedly due to the rapid progress of the pathogene in most cases, which

did not give the root tissues an opportunity to form an effective cork wall. This, however, does not satisfactorily explain the absence of cork beneath many smaller lesions in which rapid progress of the pathogene had apparently ceased. A similar phenomenon was observed in the lesioning of alfalfa roots.

The results of these experiments indicate that cork is very ineffective and probably of no significance in checking root-rot caused by *Sclerotinia trifoliorum* in alfalfa and sweet clover roots. The lessened damage at a lower soil temperature is probably due to the direct effect on the pathogene rather than to the observed inhibition of cork formation.

Studies on Overwintered Plants.

It has been previously noted that root-rotting organisms cause the greatest damage to alfalfa and sweet clover plants immediately following the winter dormancy period. One of the most important of these pathogenes, *Plenodomus meliloti*, will ordinarily attack the plants only at that time (22). Different causes might be responsible for this apparent increased susceptibility of the roots. Some changes in the tissues may result from freezing during or following dormancy, or environmental conditions in the early spring may retard cork formation or other growth processes which ordinarily function in disease resistance. In the following studies, the effect of soil temperature and moisture was determined, using different varieties

of both overwintered and artificially frozen alfalfa and sweet clover plants. Plants in the second summer of growth were also studied. The normal effect of these factors, as determined in the previously reported experiments on young plants, provided a basis for comparison.

Influence of temperature on cork formation and disease development in overwintered plants. An experiment was conducted in order to determine the effect of different controlled soil temperatures on the recovery of naturally overwintered plants. It was thought that this might reveal the relation, if any, of cork formation to disease development at that time and explain reported varietal differences in winter hardiness.

The following varieties of alfalfa and sweet clover, from the 1929 field planting, were used:

Alfalfa: Cossack and Grimm,
Sweet clover: White Blossom and Arctic.

The last named variety of each has appeared to be more hardy under the winter conditions prevailing in western Canada. Plants of these varieties were removed from the frozen soil in March, 1930, and stored at a freezing temperature until treated. The general method of treatment described was used except in the method of inoculation. A core was punched from each tap root by means of a leather punch. Pure culture inoculum of Plenodomus meliloti was then placed in this core under aseptic conditions and each end of the hole plugged with cotton batting. Each root was also cut with a sharp scalpel as a check on cork

formation. The plants were then planted in crocks of moist soil which were divided into three equal lots and held at soil temperatures of 3° C., 8° C. and 16° C. These temperatures were obtained in the two cold rooms and greenhouse, respectively, in a similar manner to that already described in the studies on young plants.

Four plants of each variety, at each temperature, were taken up at intervals of 8, 13, 23 and 28 days after treatment. All roots were preserved in form-acetic fixing solution after notes had been taken on disease development. Histological examination was made later, using the general cutting and staining methods described.

The data obtained on cork formation is presented in Table IV. No cork was formed during the course of the experiment in the roots of the plants held at a soil temperature of 3° C. At 8° C. cork was not formed until 23 days after treatment and only in the alfalfa roots at that time. In 28 days, a broken cork layer or at least the formation of a phellogen layer was observed in all roots. At 16° C. a broken layer of cork, 1 cell thick was formed in 13 days. This layer was fairly regular and 1 - 2 cells thick in 28 days. It was more pronounced in the alfalfa, than in the sweet clover roots. More cork was generally formed in the non-inoculated than in the inoculated portion of a root. No significant varietal differences in cork formation were observed.

The development of disease in the roots is shown in Table V.

Table IV. Effect of different soil temperatures[a] on wound cork formation in the roots of overwintered alfalfa and sweet clover, non-inoculated and inoculated with Plenodomus meliloti.

Variety	Treatment	Cork Rating[b] at Different Periods after Treatment											
		8 days			13 days			23 days			28 days		
		3°	8°	16°	3°	8°	16°	3°	8°	16°	3°	8°	16°
Grimm Alfalfa	Inoculated	0	0	0	0	0	2.3	0	0	3.5	0	0.1	5.7
	Non-inoculated	0	0	0	0	0	3.7	0	0.5	3.0	0	0.7	5.8
Cossack Alfalfa	Inoculated	0	0	0	0	0	0.6	0	0.1	2.0	0	0.3	3.0
	Non-inoculated	0	0	0	0	0	2.1	0	1.0	5.0	0	0.7	4.5
White Blossom Sweet Clover	Inoculated	0	0	0	0	0	2.1	0	0	2.6	0	0.2	2.5
	Non-inoculated	0	0	0	0	0	1.0	0	0	3.7	0	1.1	3.0
Arctic Sweet Clover	Inoculated	0	0	0	0	0	2.6	0	0	3.4	0	0.5	3.5
	Non-inoculated	0	0	0	0	0	2.3	0	0	4.0	0	0.6	4.7

a. Soil temperature in degrees Centigrade.

b. Average numerical rating of four replicates.

Table V. Development of *Plenodomus meliloti* in the roots of overwintered alfalfa and sweet clover plants kept at different soil temperatures[a].

Variety	Infection Rating[b] at Different Periods after Inoculation											
	8 days			13 days			23 days			28 days		
	3°	8°	16°	3°	8°	16°	3°	8°	16°	3°	8°	16°
Grimm Alfalfa	0	0.9	0	0	1.0	0.7	1.0	1.5	0.4	2.0	0.5	1.6
Cossack Alfalfa	0	1.0	0.4	0	0.8	0.2	1.0	1.2	0.2	1.6	1.8	0.8
White Blossom Sweet Clover	0	0.1	0	0	0.3	0.7	0.8	1.4	1.1	2.0	0.5	1.4
Arctic Sweet Clover	0	0.1	0	0	0.1	0	0.5	2.4	0.5	1.5	3.0	0.4

a. Soil temperature in degrees Centigrade.

b. Average numerical rating of four replicates.

Very little infection was obtained and the results were not consistent. The plants had made a rapid recovery following planting and were quite green and healthy throughout the course of the experiment, even in the lighted cold rooms. Only small root lesions were formed in any case and it would appear that conditions were not favorable for development of the pathogene. This had no relation to cork formation, since there was often no cork layer beneath these lesions and when present it was always broken and ineffective.

The temperature relations established for cork formation, in studies on the young, unfrozen plants, were mostly confirmed in this experiment. It was inhibited or retarded at the lower soil temperatures. The results indicate that cork formation was somewhat retarded in the overwintered plants, as shown by a comparison with the data for young plants in Table I. Considerably more cork was formed in the latter than in the former, in the same time, under the same conditions. Under the conditions of this experiment, cork formation has no apparent relation to disease development following the winter dormancy period.

Further support for the above conclusions was obtained from the examination of other overwintered material. This consisted of both naturally infected roots of alfalfa and sweet clover, and roots which had been inoculated in the field with Plenodomus meliloti. This field material generally showed a relatively high degree of infection and gave more

conclusive results than those obtained in the greenhouse experiment.

Overwintered roots of different varieties of alfalfa and sweet clover, showing natural infection from *Plenodomus meliloti*, have been collected and preserved for the past two or three seasons. A histological examination was recently made of this material. Abrupt checking of the progress of the pathogene was frequently noted but this was rarely accompanied by any marked degree of cork formation. A cork layer was often formed beneath lesions, but this was usually only one 1 - 2 cells thick, uneven and frequently interrupted. Differences in varietal reaction to this pathogene have been reported but no differences in cork formation were observed in the varieties examined.

Artificial inoculations had also been made in the field in connection with studies on varietal resistance to the root-rotting pathogenes. Roots of alfalfa and sweet clover had been inoculated with *Plenodomus meliloti* and other organisms after freeze-up in the fall and taken up a few weeks after thawing in the spring. Samples of this material, which had been preserved, were examined for cork formation. The invasion of *Plenodomus meliloti* was often abruptly checked in these roots with a sharp line of demarcation. Cork formation beneath these lesions was very variable in different lesions and roots, varying all the way from none to an occasionally thick cork wall. In most cases, however, the cork layer was

broken at several points and absent at the edges of the lesion. Generally it appeared to be very ineffective as a barrier to pathogene invasion.

These studies indicate that cork formation is apparently not responsible either for the early progress or subsequent checking of the invasion of *Plenodomus meliloti* in overwintered plants. The frequent absence of an effective cork layer beneath lesions in which fungous development has ceased, supports this conclusion. The direct effect of high temperature or of some other agency on the pathogene is apparently responsible for the cessation of its progress in the tissues.

Influence of soil temperature and moisture on cork formation and disease development in artificially frozen plants.

The object of this experiment was to determine the actual effect of freezing on cork formation and disease development in alfalfa and sweet clover roots. The plants were artificially frozen under controlled conditions in order to check the results obtained with over-wintered plants. The influence of soil temperature and moisture on the plants, following thawing, was also studied.

The same varieties of alfalfa and sweet clover were used as in the experiment on overwintered plants. The plants were obtained from the 1930 field planting and were the remainder of the lot used in the controlled experiments on young growing plants. They were transplanted directly from the field into boxes of soil in one of the cold rooms. The room was lighted,

continuously and kept at a temperature of about 12° C. for one week after the plants were transplanted. They were in a vigorous healthy condition at this time and were gradually hardened off by lowering the temperature 2° to 3° C. each day.

The plants were treated just prior to freezing. Each plant was cut and also inoculated with *Plenodomus meliloti*, using the general methods described. The air temperature of the room was then immediately lowered to about - 12° C. Even at this temperature, 3 days elapsed before the soil temperature was lowered to -5° C. The frozen soil was held at approximately this temperature for 3 days. The air temperature was then raised to about 6° C. and the soil allowed to thaw. After thawing, the plants were kept in the cold room at a temperature of 10 - 12° C. for 8 days. One-half of the plants were kept under dry soil conditions and the other half under relatively moist soil conditions throughout the experiment. These soil moistures were kept as constant as possible at approximately 20 per cent and 30 per cent,respectively, of the dry soil weight, following thawing.

At the end of 8 days the first roots were removed for examination. The boxes were then taken into the greenhouse and held at a soil temperature of about 18° C. for another 15 days. Plants were also taken up for examination at periods of 13 days and 23 days after treatment. All roots were preserved and later examined by the general histological methods described.

Table VI. Effect of soil moisture and temperature on wound cork formation in the roots of artificially frozen alfalfa and sweet clover, non-inoculated and inoculated with Plenodomus meliloti.

Variety	Treatment	Cork Rating[a] at Different Periods after Treatment					
		8 days[b]		13 days		23 days	
		Dry	Moist	Dry	Moist	Dry	Moist
Grimm Alfalfa	Inoculated	0	0	0	0.4	0	0
	Non-inoculated	0	0	3.2	1.4	2.8	0.5
Cossack Alfalfa	Inoculated	0	0	0	0.5	0	0
	Non-inoculated	0	0	4.0	2.0	5.5	3.2
White Blossom Sweet Clover	Inoculated	0	0	1.5	0.5	2.0	1.3
	Non-inoculated	0	0	3.8	1.2	6.4	6.8
Arctic Sweet Clover	Inoculated	0	0	1.0	0.8	1.2	1.2
	Non-in-culated	0	0	3.8	1.5	8.0	6.5

a. Average numerical rating of four replicates.
b. Soil temperature was raised from 10°C. to 18°C. after 8 days.

The amount of cork formed at the end of different periods is shown in Table VI. A broken layer, not more than 1 cell thick, was observed under most of the non-inoculated cuts in 13 days. This had generally increased to a regular layer 1 - 2 cells thick in 23 days, but results were very variable. Slightly more cork was again formed under dry soil conditions. Varietal differences were observed but these were not constant enough to be significant. At the end of the experiment, however, there was decidedly more corking in the sweet clover than in the alfalfa roots. The most striking feature of the experiment was the marked retardation of cork formation in inoculated roots as compared to non-inoculated roots. This was most

noticeable in alfalfa, where no cork was observed beneath lesions, except a slight trace in the moist soil at 13 days. It did not consist in any case of more than a few scattered cells.

Table VII. Development of Plenodomus meliloti in the roots of artificially frozen alfalfa and sweet clover plants kept at different soil moistures.

Variety	Infection Rating[a] at Different Periods after Inoculation.					
	8 days		13 days		23 days	
	Dry	Moist	Dry	Moist	Dry	Moist
Grimm Alfalfa	2.4	1.5	3.2	4.0	1.1	4.3
Cossack Alfalfa	3.0	3.5	3.3	4.6	1.4	3.4
White Blossom Sweet Clover	1.2	2.1	2.2	2.5	2.1	5.2
Arctic Sweet Clover	0.9	1.6	1.9	1.8	1.6	3.0

a. Average numerical rating of four replicates.

The extent of disease development is indicated by the data presented in Table VII. Small lesions were formed on practically all roots in 8 days after inoculation. They were slightly larger in 13 days, but made no further progress. Slightly more infection generally occurred in the moist soil. Arctic sweet clover appeared to be slightly more resistant than the White Blossom variety. The lesioning was more evident in alfalfa than in sweet clover roots.

These results confirm most of the findings of the previous experiments with overwintered plants. Cork formation is apparently retarded by freezing the roots. It again showed

little, if any, relation to disease development as indicated by the complete absence or presence of broken ineffective cork layers only, beneath lesions. The higher greenhouse soil temperatures apparently had a direct retarding effect on the progress of the pathogene.

Cork formation and disease development in plants of the second year's growth. The object of this experiment was to determine any differences in cork formation and disease reaction in roots of older plants of the second year's growth, as compared to that in the younger plants. Both alfalfa and sweet clover make a very rapid, vigorous growth during the second season. Owing to the biennial habit of sweet clover, the plants die following blossoming in the second summer, while the roots of alfalfa become very tough and woody at this time. The relative value of different methods of inoculation was tested in this experiment. It was conducted during mid-summer, at which time the prevailing soil temperatures were considerably higher than those obtained in the other field experiments.

Roots of the second year's growth of Grimm and Liscombe alfalfa and White Blossom and Arctic sweet clover, growing under normal field conditions, were used. The plants were inoculated on June 20th, using Sclerotinia trifoliorum and Plenodomus meliloti but no infection was obtained with the latter. Three different methods of inoculation were tried. In the core method a small core was cut through the tap root

with a leather punch, after which the inoculum of the fungus was placed in the hole under aseptic conditions. In the contact methods, the inoculum was placed against the side of the tap root, which was cut with a scalpel in one case and left uninjured in the other. Other roots similarly wounded, but not inoculated, were used as checks on cork formation. An continuous record of the soil temperature was not kept, but frequent determinations indicated that it averaged about 22° C.

The plants were all left undisturbed in the field for two months after treatment. At the end of that time the sweet clover plants, inoculated with Sclerotinia trifoliorum, were practically all dead and presented a striking contrast with the other still green and healthy plants. Notes were taken on disease development in all plants and four from each variety and each treatment were preserved for histological examination. They were cut and stained by the general methods described.

The data presented in Table VII show that decidedly less cork was formed in wounded roots of the second year's growth of sweet clover than in the young plants, even when the former were subjected to the high soil temperatures of midsummer. There was also less formed than in the overwintered roots in the spring. This indicates that cork formation in the tissues is retarded as the plants approach maturity. Abundant cork was formed in the alfalfa roots, when taken up two months after treatment, but no data was obtained as to the rapidity of its formation.

Table VIII. Cork formation in second year growth of alfalfa and sweet clover roots wounded by different methods and non-inoculated and inoculated with *Sclerotinia trifoliorum*.

Variety	Treatment	Cork Rating[a] in Different Types of Wounds		
		Core Removed	Surface Wound	No Artificial Wound
Grimm Alfalfa	Inoculated	6.5	b	
	Non-inoculated	7.1		
Liscombe Alfalfa	Inoculated	7.5	8.0	7.5
	Non-inoculated	7.2	8.5	
White Blossom Sweet Clover	Inoculated	4.7	0	0
	Non-inoculated	5.7	4.5	
Arctic Sweet Clover	Inoculated	3.0	0	0
	Non-inoculated	4.5	3.6	

a. Average numerical rating of four replicates.
b. No data obtained.

Table IX. Development of *Sclerotinia trifoliorum* in second year growth of alfalfa and sweet clover plants inoculated by different methods.

Variety	Infection Rating[a] in Different Inoculations		
	Core Inoculation	Contact Cut	Contact Uncut
Grimm Alfalfa	0.5	b	
Liscombe Alfalfa	2.0	1.2	0.7
White Blossom Sweet Clover	7.2	10.0	10.0
Arctic Sweet Clover	6.0	10.0	10.0

a. Average numerical rating of ten replicates.
b. No data obtained.

Sweet clover was very severely attacked by Sclerotinia trifoliorum, while alfalfa again proved quite resistant, as shown in Table IX. The core treatment proved to be the least effective method of inoculation. Sweet clover roots inoculated by this method were badly rotted, but the plants were generally alive. All sweet clover plants were dead with completely rotted roots, where the contact methods of inoculation were used. This indicates that the fungus was capable of penetrating the normal cork wall of the uninjured tap roots.

Only very slight lesions were formed on the inoculated alfalfa roots which completely confirmed the previous results obtained from inoculation of alfalfa with Sclerotinia trifoliorum. A layer of cork cells 2 - 3 cells thick, and sometimes even two separate layers were generally formed beneath these lesions. This was the only case in the entire investigation in which cork formation appeared to be effective in walling out fungous invasion.

Cork formation, however, cannot be the only cause of disease resistance in alfalfa roots because a similar checking of fungous invasion was observed in the other field experiments, when no cork was formed beneath the lesions. It would appear that cork formation in alfalfa roots was stimulated by the high soil temperature conditions of this experiment and may have aided in checking the progress of the pathogene. It is, however, probably not the fundamental cause of disease resistance in these plants.

General Histological Studies.

Histological features of normal alfalfa and sweet clover roots at different stages of growth. A study of the normal histological characteristics of alfalfa and sweet clover roots has been made in connection with this investigation. This was essential to a proper understanding of cork formation and disease development in the different tissues and at different stages of growth. The development of the root tissues was studied by growing seedlings in the greenhouse and taking up plants every two weeks for examination, in the early stages of growth. Plants in all the later stages of growth were examined at some time during the course of the above experiments.

The histological development and characteristics of alfalfa and sweet clover root tissues were found to be essentially the same. No differences worthy of mention were observed and the following general description is applicable to both.

The roots were first examined shortly after emergence of the cotyledons. These very young roots consisted essentially of a well defined stele in the centre, bounded by a fairly wide cortex, outside of which was a loose layer of cells.

Secondary growth commences shortly after the first few trifoliate leaves of the seedling have become expanded. The cambium cylinder arises from the pericycle at this time and begins producing secondary phloem on the outside and secondary xylem on the inside. The outer portion of the root is at first covered by the primary cortex, but this becomes stretched and

ruptured by secondary growth and eventually sloughs off. The phellogen or cork cambium then arises from the pericycle and forms a periderm or cork layer externally and a phelloderm internally. The normal cork formed acts as a protective layer and surrounds all other root tissues. The cork layer was complete in roots 4 weeks old, but many of the ruptured cortical cells were still adhering to the outside and traces of these were often observed in older plants.

When secondary growth is complete, the normal cork layer, which occurs at the outside, is usually 3 - 4 cells wide and very regular. Additional cork layers, usually not more than 2 cells wide, extend beneath the points at which branch roots pass out. Beneath the cork layer lie several layers of irregularly arranged parenchymatous cells, below which occurs the secondary phloem. This consists of sieve tubes and companion cells intermingled with numerous groups of schlerenchymatous fibres. The cambium layer of actively dividing cells separates the phloem from the xylem and completely encircles the root. The wood cells of the xylem are large and thick walled. They are not very numerous and are irregularly arranged, being separated by parenchymatous cells and groups of fibres. A true pith is usually absent since the xylem extends nearly to the centre of the root.

The occurrence of groups of fibres in practically all tissues is one of the most characteristic features of these roots. These fibres have thick, lignified walls and proceed

in a sinuous manner through the tissues. Their inability to form cork makes them of special significance in this study.

Manner of wound cork formation in the different root tissues. The wounding of a root by cutting was generally followed by a definite sequence of tissue changes. The first sign of healing which occurred was the knitting together of the ruptured cells at the cut surface. A distinct dark line was often formed, apparently caused by the deposit of some wound substance in the surface cells. This dark substance was generally more evident in the wounds of roots kept under moist conditions than under dry conditions, and was usually associated with less cork formation. It did not respond to staining tests for suberin. The observed retarding of cork formation may have been due to exclusion of air from the tissues by the dark deposit. Priestley and Woffenden (20) concluded that air was necessary for the initiation of cork formation in potato tuber wounds, but they found that it was more favored by moist than by dry atmospheric conditions.

The formation of a phellogen layer in the root tissues indicated the initiation of wound cork formation. This appeared as a continuous layer of short, actively dividing cells, similar in appearance to the ordinary cambium layer. After a short time cork cells were formed on the outside of this layer. In many cases their formation appeared to be due to the drying up of the cell contents and other modifications of the normal cells in that position. A phelloderm consisting

of new parenchymatous cells was usually formed on the inside of the phellogen. Cork cells normally became suberized immediately after being formed, as evidenced by their staining a bright orange with the cork stain; Sudan III. Suberization was delayed under certain conditions, especially in moist soils.

Wound cork layers were normally formed in a position parallel to the surface of the wound and connected up with the edges of the normal cork layer (Fig. 1). Great variations occurred in the depth at which the cork layer was formed below the wound. Several layers of cells, however, generally separated it from the surface of the wound. The tissues between usually stained abnormally and there was an evident disappearance of their cell contents.

The different cell tissues of the root were found to possess distinctly varying potentialities for cork formation. The studies of Jones (12) with alfalfa were closely confirmed in this respect. Cork was generally formed most readily in the parenchymatous tissues below the normal cork layer and in the phloem. It was often somewhat delayed in the region of the cambium layer and in the xylem, but formed readily in the central parenchyma cells of the root, when these were present. Certain wood cells of the xylem sometimes did not form cork. Fibre groups were incapable of cork formation and often interrupted cork layers. Other cases were observed in which the cork layer jutted out around the fibres (Fig. 2).

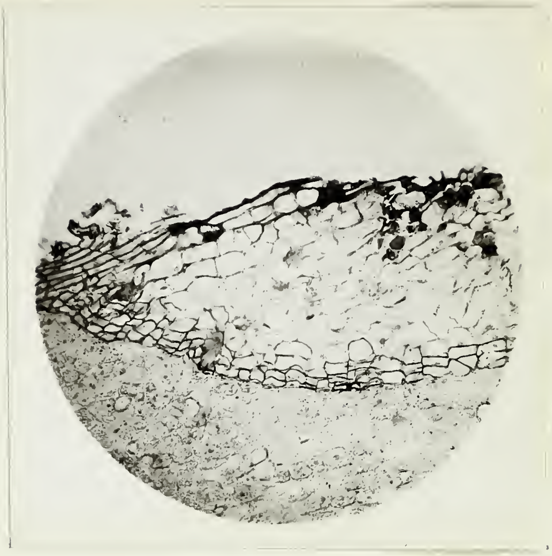

Figure 1. Wound cork formation beneath a non-inoculated surface cut in an alfalfa root 30 days after wounding. Note the manner of connection with the normal cork layer at the left. X 85.

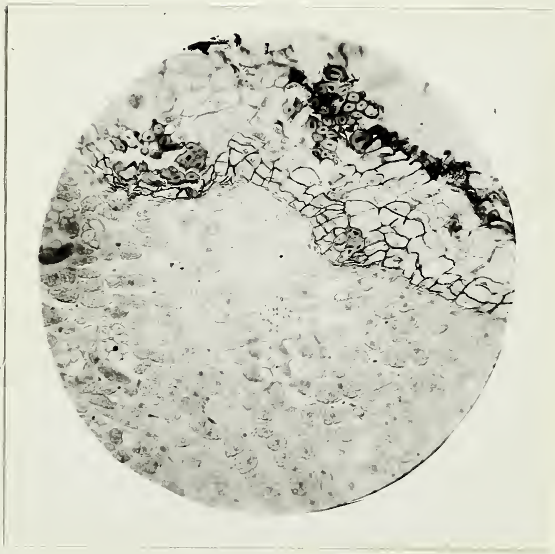

Figure 2. Interruption and jutting out of a wound cork layer beneath a surface cut, caused by the occurrence of groups of fibres in the root tissues. X 85.

The method of wounding had some effect on cork formation. Cork was generally most quickly and regularly formed in a surface cut, parallel to the axis of the root. It was often delayed and broken in deep longitudinal cuts, which may have been due to lack of air. The general features of wound cork formation have been described but many unexplained irregularities were observed.

The effect of freezing and age on cork formation in the roots has already been considered in the various experiments. Other interesting observations were also made on the effect of the condition of the root tissues on cork formation. Preliminary studies showed that cork formation did not take place when the roots were kept under non-growing conditions. Unfavorable growing conditions also seemed to have a deleterious effect. A marked retardation of cork formation was noted in plants which had been grown from seed in crocks and had been kept in the greenhouse for a year or more, under relatively unfavorable conditions for growth. In most cases cork formation was completely inhibited, or at least only scattered cells were formed, a long time after wounding. No pronounced histological abnormalities were observed in these roots.

Pathogene penetration and development in the root tissues. Various methods of inoculation were used in the different experiments in order to determine the mode of penetration of pathogenic organisms into the host root tissues. Both _Sclerotinia trifoliorum_ and _Plenodomus meliloti_, in practically

all cases, were found to cause as much infection in unwounded roots as in roots wounded at the point of inoculation. There was of course the possibility that some of the roots were accidentally injured, but the constancy of the results indicated that both pathogenes were apparently capable of penetrating the uninjured normal cork wall at the outside of the root. Histological examination has confirmed this conclusion. Preliminary observations indicate that these two pathogenes penetrate the normal cork wall and develop in the tissues in a strikingly different manner.

The mycelium of *Sclerotinia trifoliorum* is shown penetrating the normal cork wall of a sweet clover root in Figure 3. This has been observed in a number of sections from both alfalfa and sweet clover roots. These sections show that a single hyphal strand of the fungus is apparently capable of direct mechanical penetration through the cork wall, but the exact method by which this is accomplished is still obscure. There is some indication, however, of possible formation of an appressorium on the outside followed by constriction of the fungous hypha as it enters (see "a" Fig. 3). In 1886, DeBary reported the penetration of *Sclerotinia libertiania* into the leaves of broad bean by this method. It is a fairly common method of fungous penetration through ordinary cells, but no report of single hyphal penetration of cork cells has been found in the literature.

The mycelium of *Sclerotinia trifoliorum* progresses rapidly through the tissues after gaining entrance. It appears to proceed in both an intercellular and intracellular manner (Fig.4.).

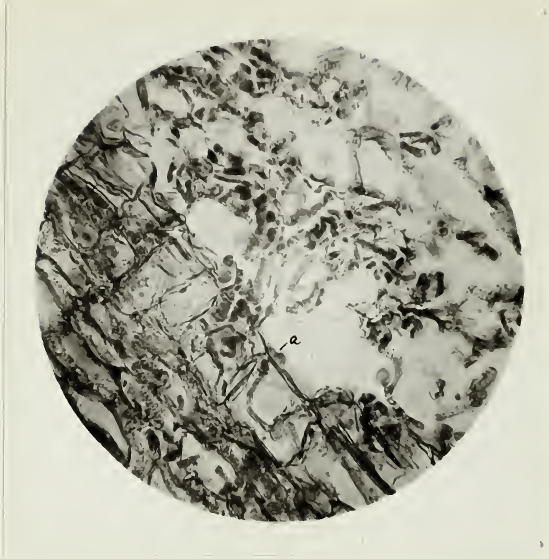

Figure 3. Penetration of the mycelium of Sclerotinia trifoliorum through the normal cork wall of a sweet clover root 10 days after inoculation. Note the apparent constriction of one fungous hypha as it enters at point "a". X 240.

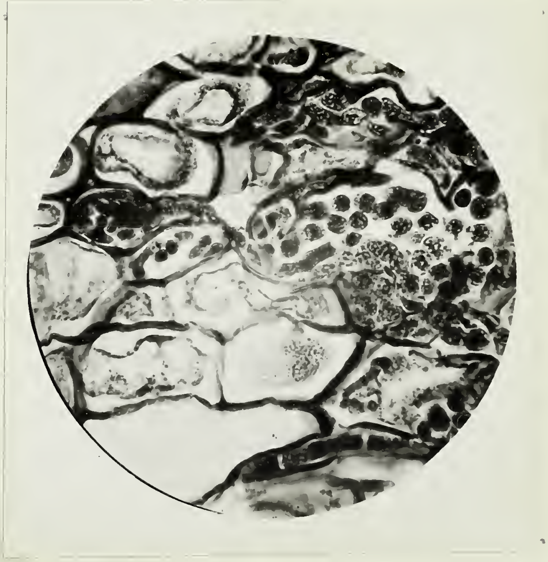

Figure 4. Progress of the mycelium of Sclerotinia trifoliorum through the cell tissues of a sweet clover root. It is shown proceeding in an intracellular manner in the centre and in an intercellular manner at the bottom of the photograph. X 350.

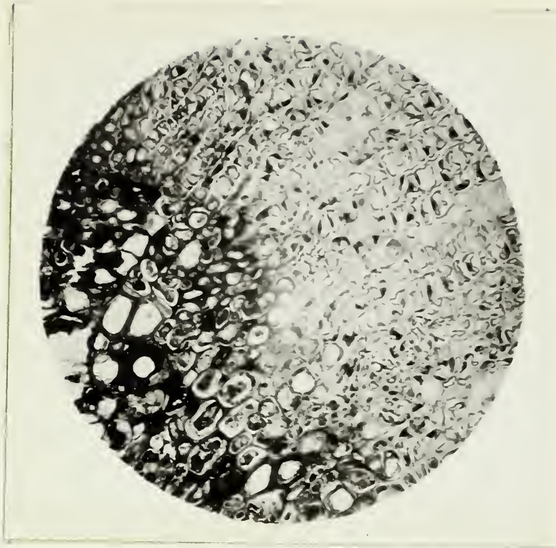

Figure 5. Unhindered progress of Sclerotinia trifoliorum through a sweet clover root showing disorganization of the cells in advance of the fungous mycelium. X 140.

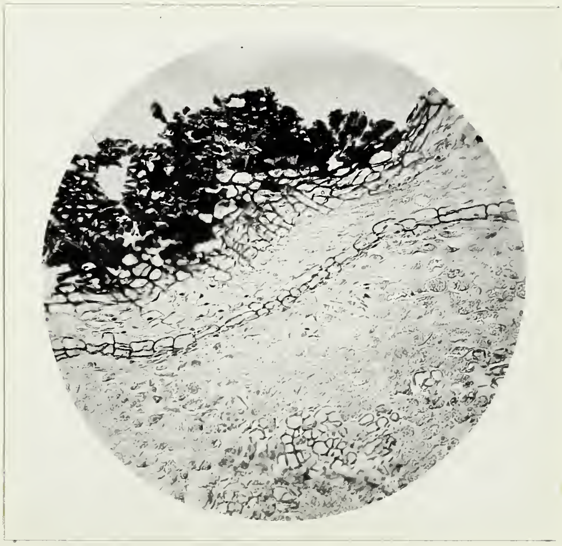

Figure 6. Invasion of Sclerotinia trifoliorum apparently walled out by cork formation in the second summer's growth of an alfalfa root. The first wound cork layer is nearly penetrated at one point but a second one has been formed deeper in the tissues. X 85.

In many cases a dense mass of hyphae appear to rupture their way through the cells by direct mechanical force. The penetration of wound cork layers in a similar manner to the original penetration of the root was also observed. The cell tissues were often disorganized ahead of the mycelium, which indicated the possible toxic action of a fungous excretion (Fig.5).

Although wound cork formation did not generally appear to hinder the progress of Sclerotinia trifoliorum in the root tissues, this was not always the case. An occasional exception was noted in the alfalfa roots inoculated during the second summer of growth, in which the pathogene was apparently walled out by wound cork. This is illustrated in Figure 6, where the pathogene has nearly penetrated the first wound cork layer at one point, but a second layer has been formed deeper in the tissues. This indicates that under high soil temperature conditions, cork formation may be a contributing factor in the resistance of alfalfa roots to invasion by Sclerotinia trifoliorum. It was also commonly noted in all experiments that saprophytes or weak pathogenes were often apparently walled out by cork formation after they had gained entrance to the root through accidental wounds. The cork layer which was formed beneath these small lesions under favorable conditions was very similar to that formed beneath non-inoculated wounds.

Plenodomus meliloti appeared to penetrate the normal cork wall by mass hyphal action. The hyphae accumulated in close contact to the cork wall at certain

points which resulted in its ultimate disintegration. (Fig. 7). Conant (7) reported a similar penetration of Thielavia basicola into tobacco roots. He believed that the cork wall was ultimately destroyed by the action of accumulated enzymes excreted by the fungus. The pycnidia of Plenodomus meliloti were sometimes found imbedded in the cork wall after the fungus had gained entrance (Fig. 8). A dense stroma, from which the pycnidia often arise, was present in other cases. (Fig. 9.)

The development of the mycelium of Plenodomus in the tissues was seldom observed. A few sections were obtained which show the hyphal strands of the fungus in close proximity to the cell walls (Fig. 10). Intercellular progress was indicated by the absence of mycelium in the cells. Damage to the cell tissues was always caused at some distance in advance of the invading pathogene. This was indicated by marked shrivelling of the cell contents, separation of the vascular bundles, and the abnormal staining properties of the affected area. This suggests the toxic action of some substance exereted by the fungus.

The penetration of wound cork layers by this fungus has not been observed. It has been noted in the above experiments that a cork layer was frequently absent beneath lesions, or if present, was usually broken and ineffective. Abundant cork was often formed beneath non-inoculated cuts under the same conditions. This suggests the possibility that cork formation

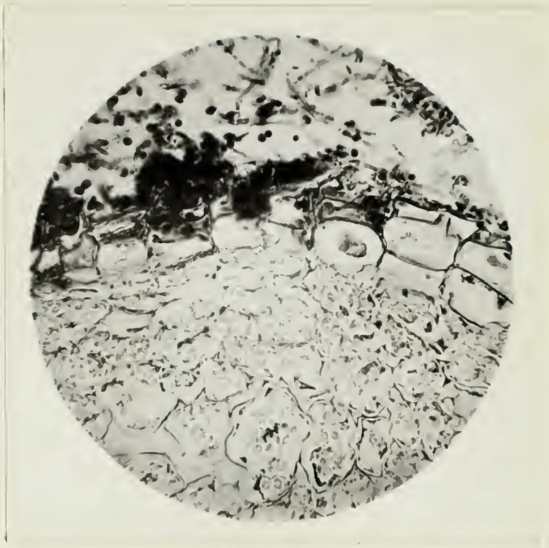

Figure 7. Penetration of the mycelium of Plenodomus meliloti through the normal cork layer of a sweet clover root. The cork cells are apparently destroyed by mass action of the fungous hyphae. X 240.

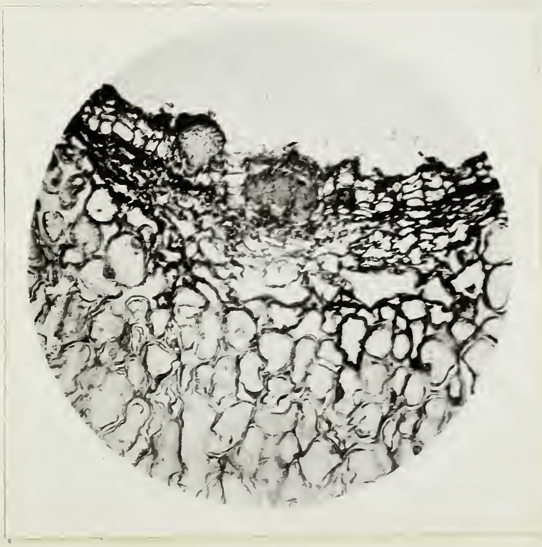

Figure 8. Formation of pycnidia by Plenodomus meliloti in the normal cork layer after it has been ruptured by the pathogene. X 140.

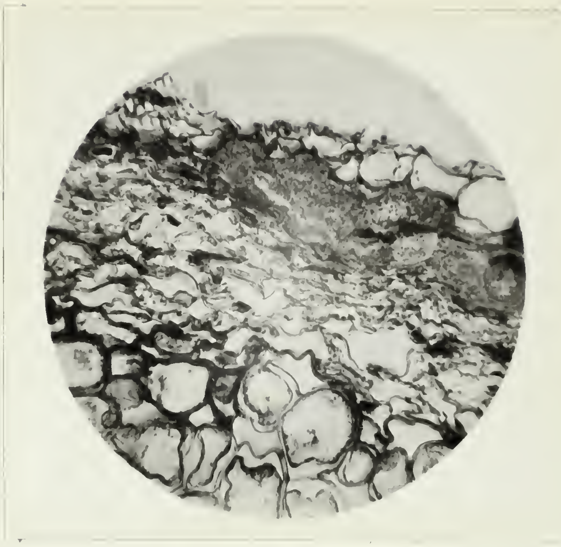

Figure 9. Formation of a dense stroma of mycelium of Plenodomus meliloti just beneath the point of penetration of the normal cork layer. X 240.

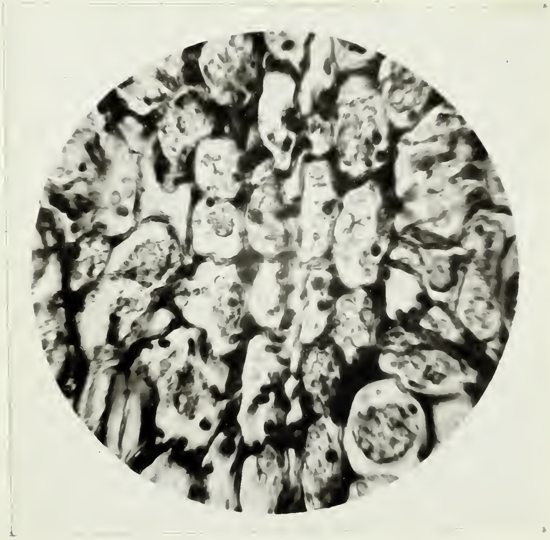

Figure 10. Progress of the mycelium of Plenodomus meliloti in an apparently intercellular manner through the tissues of a sweet clover root. Tips of the hyphae are seen projecting from the cell walls. X 365.

may be inhibited by a fungous excretion but further studies are necessary before this theory can be definitely postulated.

GENERAL DISCUSSION AND CONCLUSIONS

The results obtained in this investigation indicate that cork formation in the roots of alfalfa and sweet clover has the same general features but probably has a different significance than that reported in the roots and other parts of certain other plants. A consideration of the manner of cork formation and its influencing factors, as observed in the different experiments, may give a clearer understanding of the results obtained with regard to disease development.

All root tissues were found to be potentially meristematic in nature with the exception of the sclerenchymatous fibre groups and certain wood cells. Wound cork was most readily formed in parenchymatous tissues in different portions of the root and in the xylem. These results confirm very closely the findings of Jones (12) and indicate that the character of the cell tissues may condition their response to injuries. This was often borne out in cases where a group of fibres interrupted a wound cork layer. Any pathogenic organisms present could have readily passed through the cork wall at that point. In other cases the wound cork layer detoured around the obstructing fibres, which indicated the ready adaptability of the tissues to emergencies.

Certain environmental factors were found to have an influence

on cork formation, the most marked effect being obtained with varying soil temperatures. In general, cork formation appeared to be directly correlated with soil temperature, since it was inhibited at temperatures slightly above freezing and occurred with increasing rapidity at the higher temperatures employed in the different experiments. It would seem, however, as if cork formation was not particularly rapid under average soil temperature conditions. At 18° C. a really effective cork wall was not formed until 13 days after wounding. Under ordinary conditions this length of time would give rapidly progressing pathogenic organisms ample opportunity to invade deeper tissues. Artschwager (2) and others concluded that the rapidity with which wound cork was formed was of more importance than the thickness of the layer.

In view of the fact that slightly more wound cork was formed under dry soil than under moist soil conditions, on the average, in all experiments, this factor must be considered. Under the conditions of these experiments, however, the difference was not large enough to be of practical significance.

Investigations reported on the other plants (2, 20, 29, 30) show that cork formation is generally favored by high atmospheric humidity. The only exception found in the literature was in reported results on the wounding of leaves (4, 21, 33). None of these studies are very comparable to the present investigation, since they all refer to the effect of atmospheric humidity and not to soil moisture. It is suggested that lack of proper aeration in the moist soils was largely

responsible for the results obtained. Priestley and Woffenden (20) found that air was necessary for the initiation of wound cork formation, and other workers have indicated its importance (8, 11). The irregularity of wound cork layers formed in deep root cuts as compared to that in surface cuts also indicated an air relation.

No satisfactory explanation can be offered for the dark wound substance often formed in the surface cells of cuts. This did not prove to be suberin which has been reported as forming in the surface cells of wounds in other plants (2, 20, 29). It was usually associated with moist soil conditions and a slight retardation of cork formation.

Cork formation was also found to be influenced by the age and condition of the roots. It seems to be somewhat retarded by freezing the root tissues, either artificially or in natural overwintering. This effect is probably due to some influence of frost on the tissues, since it was also evident in young artificially frozen roots. It would appear that the general vigor of the plant has a marked influence on cork formation, which may also be a possible explanation of the effect of freezing. Cork did not form in the tissues of non-growing roots and was greatly retarded in the roots of plants growing under adverse conditions in the greenhouse.

The ability of the root tissues to form cork readily is apparently lessened as the plants approach maturity. This is indicated by the distinct retardation of cork formation in sweet clover roots of the second summer's growth. Alfalfa

roots of the same age were found still to form cork quite readily, but there might possibly be a retardation in succeeding years of growth.

Significant varietal differences in cork formation were not found to occur in either alfalfa or sweet clover. There was, however, considerable variation in results which often differed in the different experiments. This may have been due to the influence of other factors. At this point, however, the apparently high degree of natural variability occurring in individual roots of alfalfa and sweet clover may be emphasized. Variations in cork formation often occurred in the roots of plants receiving identical treatment, which could only be explained on the basis of natural variability.

The two pathogenic root-rotting organisms studied in connection with this problem exhibited a marked contrast in practically all characteristics and will be considered in detail separately.

Sclerotinia trifoliorum was found to cause serious damage in the field at any time during the growing period, particularly to sweet clover. Its progress through the root tissues was, however, somewhat retarded by low soil temperatures. Artificial infection has seldom been obtained under greenhouse conditions, for reasons which are not clearly understood.

Under field conditions all varieties of alfalfa showed marked resistance to the strain of the pathogene used. This was isolated from a diseased sweet clover root and other isolations have not as yet been tested on alfalfa. Small

lesions were formed on the roots in a short time after inoculation, but these rarely developed any further. This resistance of the root tissues to further invasion was apparently not associated with cork formation, since a very ineffective cork layer was usually formed beneath such lesions. It would appear to be due to some form of physiological resistance in the tissues.

Sweet clover on the other hand was very severely attacked by this pathogene and death of the plant often resulted in a few days after inoculation. In this case cork formation apparently offered no barrier to the progress of the invading pathogene. This was probably due to the rapidity of the invasion in most cases, which did not give the tissues an opportunity to initiate cork formation, or at least to form an unbroken layer. In a few cases, where formed, it was apparently penetrated by the pathogene.

The White Blossom variety of sweet clover was found to be somewhat more susceptible to the attack of this pathogene than the other varieties tested. As in the case of alfalfa, this did not appear to be correlated with cork formation and some other form of resistance was probably responsible.

Evidence was obtained which indicates that *Sclerotinia trifoliorum* is apparently capable of penetrating both normal and wound cork layers in alfalfa and sweet clover roots. Indications that the normal cork layer was penetrated were obtained when it was found that the same amount of infection

always resulted when either injured or uninjured tap roots were inoculated. The normal cork wall, which constitutes the outer layer of the root, is generally very thick and regular and appears to be as an effective a barrier, if not more so, than any wound cork formed in the roots. Preliminary observations indicated that a single fungous hypha was probably capable of direct mechanical penetration through cork layers. Further studies are necessary before this can be definitely confirmed. A search of the literature has revealed no previous reports of this method of cork wall penetration.

Within the tissues, the mycelium of this pathogene appeared to proceed in both an intercellular and intracellular manner. The cells of the root were also observed to be disorganized at some distance in advance of the mycelium, which suggests the possible excretion of a toxic substance by the fungus in advance of its invasion.

Plenodomus meliloti is another root-rotting organism which is very pathogenic to alfalfa and sweet clover. It was, however, found to cause significant damage only following overwintering or artificial freezing of the roots. This was probably due either to the low temperature prevailing after thawing, or to some effect of freezing on the root tissues, which made them more susceptible to fungus attack. The development of the pathogene in the tissues was checked at high soil temperatures and apparently favored by moist soil conditions. Alfalfa and sweet clover were similarly attacked

and no striking varietal differences occurred, although Arctic sweet clover was generally more resistant than the White Blossom variety.

The observed increase in susceptibility of the roots to attack after freezing was not in any way associated with cork formation. It is true that cork formation would be retarded at the low soil temperatures prevailing at that time, but it was found not to be responsible for the arresting of the progress of the pathogene at higher soil temperatures. This was indicated by the frequent absence or presence of only a broken cork layer beneath lesions, which were not being extended any further.

Preliminary observations indicated that this pathogene was also capable of penetrating the normal cork wall. In this case it appeared to be a mass hyphal action which resulted in the ultimate disintegration of the cork layer. Further studies are, however, necessary to confirm this and to determine the exact mode of penetration. The apparent ease of penetration is indicated by the fact that wounding of the root in inoculation was not necessary in order to induce artificial infection.

The mycelium of Plenodomus meliloti was very seldom observed within the invaded root tissues. It appeared to progress in an intercellular manner when seen. The most striking feature of infection with this pathogene was the marked disorganization and destruction of the root cell tissues in the apparent absence of mycelium, at least for a considerable distance. This suggests that the fungus may excrete a substance which has a

toxic effect on the cells.

The absence or retardation of cork formation beneath lesions caused by this pathogene was a common phenomenon. Under the same conditions and often in the same root, a well formed cork layer would often be present beneath non-inoculated wounds. This indicates the possibility that some toxic excretion of the fungus may inhibit or retard cork formation. As far as can be determined, this has never been previously reported and further studies are necessary before any definite conclusion can be reached.

The evidence obtained in this investigation indicates that cork formation is in general of little, if any, importance in relation to disease development. It did not appear to be responsible for arresting the development of the pathogene, in either resistant or susceptible plants. This was true under both laboratory and field conditions and in several different types of plants.

There still remains the possibility, however, that cork may be a contributing factor in disease resistance at relatively high soil temperatures, probably higher than under normal field conditions. This was indicated by the occasional apparent walling out by cork formation of Sclerotinia trifoliorum infection in alfalfa roots under mid-summer conditions in the field. This investigation, therefore, does not prove definitely that cork formation is not a factor in root-rot resistance in alfalfa and sweet clover roots, under certain conditions.

Cork formation is apparently of significance in checking

the invasion of weakly parasitic fungi. They seldom cause damage, however, and are of little practical importance.

SUMMARY.

1. Cork formation in relation to root-rot development in alfalfa and sweet clover has been studied in both young and over-wintered plants of different varieties under controlled conditions of soil temperature and moisture in the laboratory, and under natural field environment.

2. Histological examination shows that cork formation is directly correlated with soil temperature. Cork was formed in 8 days at 18° C. and in 18 days at 9° C. but was absent even in 30 days at 1° C.

3. Slightly more cork was formed in dry than in moist soils but the difference is probably of no practical significance.

4. Cork formation was retarded in the roots of over-wintered or artificially frozen plants, of plants grown under unfavorable conditions in the greenhouse and of the second summer's growth of sweet clover.

5. No significant varietal differences in cork formation occurred.

6. The root rotting pathogene Sclerotinia trifoliorum progressed very rapidly in the root tissues of sweet clover under

favorable conditions. It was somewhat retarded by low soil temperatures.

7. The root tissues were apparently incapable of forming an effective wound cork layer in time to act as a barrier to the rapidly invading pathogene. In other cases it appeared to penetrate the cork layer formed.

8. Alfalfa was markedly resistant to *Sclerotinia trifoliorum* and Arctic sweet clover was less susceptible than other varieties tested. These differences in disease reaction may probably be attributed to some form of physiological resistance in the tissues, since cork formation was normally retarded beneath lesions in which progress of the pathogene had ceased.

9. A recently described root rotting pathogene, *Plenodomus meliloti* caused serious damage only following freezing of the roots, either artificially or in natural over-wintering. It was always retarded and often inhibited at high soil temperatures.

10. Cork layers were usually absent or retarded beneath lesions produced by the pathogene. The direct effect of high soil temperature or other environmental factors on the pathogene seemed to be the chief reason for retardation of its progress.

11. Both pathogenes were found to attack unwounded roots readily. Evidence was obtained that they are capable of penetrating the normal cork wall.

12. The root cell tissues were commonly disorganized at a considerable distance in advance of the mycelium of the invading pathogene, particularly in the case of *Plenodomus meliloti*. This may have been due to the toxic action of some substance excreted by the fungi.

13. The results, in general, indicate that cork formation has no significance in relation to the development of these pathogenes in either alfalfa or sweet clover roots. It appears to check the invasion of weakly parasitic fungi and may be a contributing factor in resistance to pathogenes under high soil temperature conditions.

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